Head Length Dimorphism of Mammalian Spermatozoa.

By

A. S. Parkes, B.A. (Cantab.), Ph.D.,

Department of Zoology, University of Manchester.

With 3 Text-figures.

Contents.

Introductory.

Work on mammalian spermatogenesis has in a large number of cases shown that the spermatozoa are of two types, one type possessing the accessory chromosome, whilst the other type has no sex chromosome or a mere vestigal complement. As the spermatozoon head is constituted almost entirely of nucleus it might be expected that the additional chromatin possessed by the one type would slightly increase the size of the head. In three cases this correlation has been found. Wodsedalek (**5** and **6**) has shown that in the horse and bull the spermatozoa are of two types, and that in each case a frequency polygon of the head lengths shows distinct dimorphism. In the case of the dog, Malone (**2**) found an unpaired accessory chromosome in spermatogenesis, and

Zeleny and Faust (**8**) have demonstrated dimorphism of the head lengths. The work recorded in this paper was an attempt to extend the application of this correlation to other mammalian spermatozoa for which chromosomal dimorphism has been shown.

Methods and Material.

The new work recorded here deals with man, the rat, the cat, and the mouse. I have to thank Mrs. R. Sellars of the Manchester Medical School for procuring the human material for me from the Manchester Royal Infirmary. In the other cases the material was obtained by dissection of the epididimis. In each case smear slides were made, as this method has advantages compared with using testis sections. Some difficulty was at first experienced in making satisfactory smears owing to the tendency for the spermatozoa to drop off. Increased experience in manipulation, however, was found to surmount this. By teasing out the epididimis in salt solution and fixing, it was found possible to make the spermatozoa adhere without using egg-albumen cement. For fixing Zenker's fluid was used to start with, as recommended by Zeleny and Faust (**8**), but finally the ordinary corrosive and aceti-solution (90 per cent. saturated solution corrosive sublimate and 10 per cent. glacial acetic acid) was found to be quite efficient. Various stains were tried, but Delafield's haematoxylin was eventually found to be by far the most satisfactory.

The measurement of the spermatozoa was found to present great difficulty, especially in the case of the rat and mouse where the head-piece is sickle-shaped. This fact, together with the minute size of mammalian spermatozoa, makes measurement with an ocular micrometer almost impossible. Both these difficulties were alleviated by using the Zeiss-Greil drawing apparatus possessed by the department. This consists of a lantern throwing light through a horizontal photo-microscope and projecting the image on to a screen. By placing a mirror at 45 degrees in front of the eye-piece the image can be thrown down on to a table. This apparatus can be used

with an oil immersion, and the resulting image thrown on the table, even of such a small object as a spermatozoon head, is sufficiently large to admit of measuring round a curve with a pair of compasses. The co-efficient was worked out previously by putting a stage micrometer in the microscope and finding out how many centimetres on the table corresponded to 10μ on the slide. The subsequent calculation was as easy as that necessary when using an ocular micrometer. By this means the unavoidable margin of error in the measurements was very greatly reduced. On one occasion when the drawing apparatus was out of order some measurements were made with the aid of a camera lucida. In both cases extreme dimensions were marked on paper, connected by a line, and the whole number measured afterwards. An 18 Zeiss ocular could be used with the camera lucida, but not with the drawing lantern, owing to the excessive diffusion of light, and so the nett magnification came to about the same in both cases.

Wodsedalek measured the spermatozoa in testis sections by the camera-lucida method, and Zeleny and Faust used smear slides with the ocular micrometer. It will be seen, therefore, that the method described above is a combination of the two, and it was found to be the most satisfactory. The error involved by the use of the ocular micrometer is avoided, as is the possibility of getting immature spermatozoa on the slide.

As the whole value of the work depends on the degree of accuracy which can be achieved, the following remarks may not be out of place. Three sources of error arise :

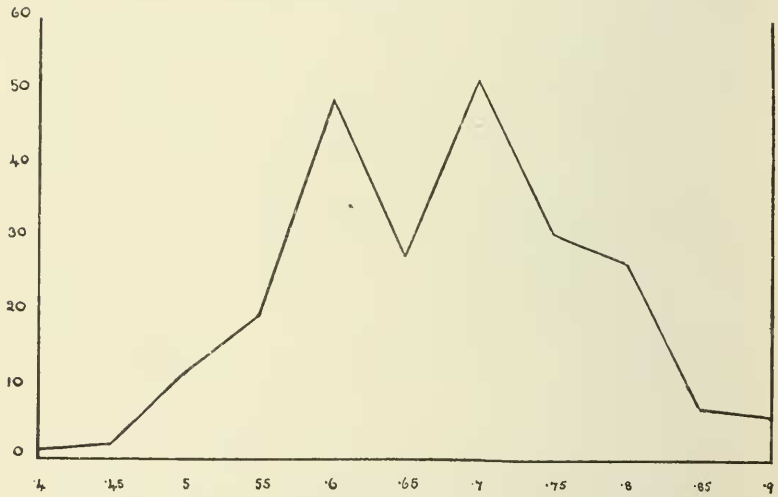
(*a*) Distortion of the spermatozoa during fixing and preparing.
(*b*) The personal factor in measuring.
(*c*) The unavoidable error in measuring.

With regard to the first point, general shrinkage of the spermatozoon head must almost inevitably occur ; but only one slide was used for each set of measurements, and no attempt has been made to mix measurements from different slides. As the spermatozoa on one slide would all be affected in the

same manner, this precaution should remove the first source of error.

Secondly, bias particularly easily arises in such work and may almost unconsciously detract from the accuracy. A conscious attempt to discount this bias may lead to the opposite extreme and cause an equal inaccuracy. Also, the work is very trying and strain seriously disturbs the measurements. In general, however, the personal factor was discounted, as far as possible, by only working for very short periods at a time.

Text-fig. 1.



Frequency polygon of head lengths of human spermatozoa.

Thirdly, the unavoidable margin of error must be considered. Fortunately, however, this is a calculable quantity, and the following tests were made. In the first case the same spermatozoon head was measured under three different magnifications, the co-efficients of which were known, and the results compared. The following table sums up the results:

Ob. and Oc.	*Size in μ.*
Bausch and Lomb $\frac{1}{6}''$ and Zeiss 18 .	4·09
Koristka $\frac{1}{12}''$ and Bausch and Lomb 10	4·10
Koristka $\frac{1}{12}''$ and Zeiss 18 . . .	4·14

It will be seen that the range of the variation in the three measurements is only $0{\cdot}05\mu$ or $\frac{1}{20}\mu$.

The second manner of testing accuracy consisting in measuring the same spermatozoon several times under the same magnification, and the results gave a range of variation in six measurements of $0{\cdot}10\mu$ or $\frac{1}{10}\mu$. Both these margins of error are very much smaller than the least fraction of μ usually dealt with.

I should like to take this opportunity of acknowledging my great obligation to Mr. J. T. Wadsworth for his invaluable assistance in the technique of this work.

Spermatozoa of Man, Rat, Cat, and Mouse.

Von Winiwarter (4) has described chromosome dimorphism of human spermatozoa according to the presence or absence of an unpaired accessory. In my material, measurement of the head lengths was found to give the following results :

Table I. Frequency of Head Lengths of Spermatozoa of Man.

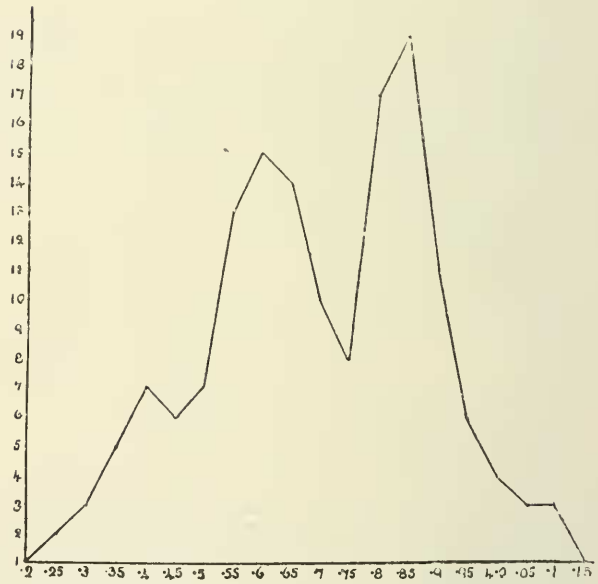
Head lengths ×1,460 (*in cms.*)	*Number found.*	*Head lengths* ×1,460 (*in cms.*)	*Number found.*
0·4	1	0·7	52
0·45	2	0·75	31
0·5	12	0·8	27
0·55	20	0·85	7
0·6	49	0·9	6
0·65	28		

In the case of the rat Allen (1) has demonstrated an accessory chromosome in spermatogenesis, the spermatozoa having eighteen or nineteen chromosomes. The dimorphism again appears to communicate itself to the head sizes of the spermatozoa, for dimorphism was found in the head lengths of the spermatozoa of the rat. The frequency polygon given below (Text-fig. 2) was made from measurements of the head lengths (× 4,000) of 155 spermatozoa.

Table II. Frequency of Head Lengths of Spermatozoa of Rat.

Head lengths ×4,000 (*in cms.*)	*Number found.*	*Head lengths* ×4,000 (*in cms.*)	*Number found.*
3·2	1	3·7	10
3·25	2	3·75	8
3·3	3	3·8	17
3·35	5	3·85	19
3·4	7	3·9	11
3·45	6	3·95	6
3·5	7	4·0	4
3·55	13	4·05	3
3·6	15	4·1	3
3·65	14	4·15	1

Text-fig. 2.



Frequency po'ygon of head lengths of spermatozoa of rat.

Von Winiwarter (**3**) has shown that the spermatozoa of the cat are cytologically dimorphic, one type having eighteen chromosomes and the other seventeen. In measuring the head lengths, however, no clear dimorphism was found. The results were as follows :

Table III. Frequency of Head Lengths of Spermatozoa of Cat.

Head lengths ×4,000 (*in cms.*)	*Number found.*	*Head lengths* ×4,000 (*in cms.*)	*Number found.*
1·2	2	1·7	32
1·25	2	1·75	20
1·3	4	1·8	28
1·35	2	1·85	13
1·4	7	1·9	22
1·45	9	1·95	6
1·5	17	2·0	5
1·55	20	2·05	1
1·6	31	2·1	1
1·65	26	2·15	1

Yocum (7) has shown that the spermatozoa of the mouse are cytologically dimorphic, one type having nineteen and the other twenty chromosomes. I found this reflected in the head lengths, as the following results show:

Table IV. Frequency of Head Lengths of Spermatozoa of Mouse.

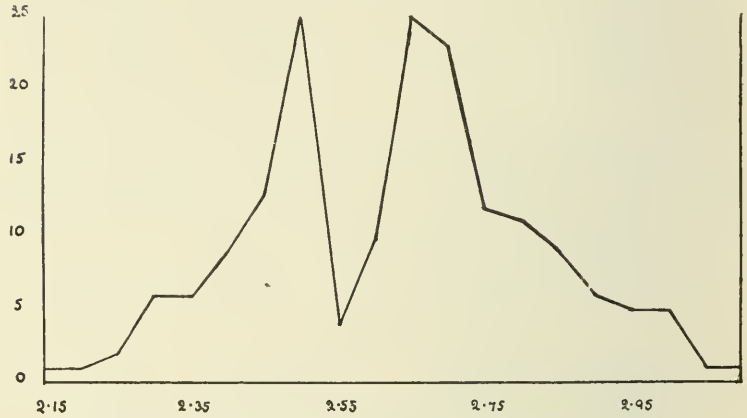
Head lengths ×4,000 (*in cms.*)	*Number found.*	*Head lengths* ×4,000 (*in cms.*)	*Number found.*
2·15	1	2·65	25
2·2	1	2·7	23
2·25	2	2·75	12
2·3	6	2·8	11
2·35	6	2·85	9
2·4	9	2·9	6
2·45	13	2·95	5
2·5	25	3·0	5
2·55	4	3·05	1
2·6	10	3·1	1

Conclusion.

It would thus appear that in three more species of mammals the spermatozoa show dimorphism in the head length, while in a fourth species, the cat, the evidence is uncertain. The chief interest in these conclusions lies in the bearing which the size dimorphism of the spermatozoa might have in determining the proportion of the sexes at conception. If the potentially male and the potentially female-producing spermatozoa are of different size, their activity and vigour may also be relatively

different, causing more of one type than of the other to survive the severe journey through the female organs to the ova (as T. H. Morgan has suggested, ' Physical Basis of Heredity ', 1919). It is most probable that the disproportion which exists between the sexes at conception in most mammals is connected with this point.

Text-fig. 3.



Frequency polygon of head lengths of spermatozoa of mouse.

Summary.

1. Chromosome dimorphism of the spermatozoa has been shown for a variety of mammals, and in some cases this has been shown to be correlated with dimorphism in the head lengths of the spermatozoa.

2. In the present paper this correlation has been extended to the spermatozoa of man, the mouse, and the rat, in which chromosome dimorphism of the spermatozoa had previously been shown, and in which head length dimorphism seems to exist.

3. The interest of these results lies in the probability that the histological difference in the X- and Y-spermatozoa may account for the inequality of the sexes at conception in mammals.

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